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DISTRIBUTION OF NORDITERPENE ALKALOIDS IN TALL LARKSPUR PLANT PARTS THROUGH THE GROWING SEASON

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Abstract—Previous research showed that toxic and total alkaloid pools in tall larkspur (*Delphinium barbeyi*) increased during early growth, then declined precipitously during the late flower and pod stage of growth. The objective of this study was to measure the concentration and pools of toxic and total alkaloids in tall larkspur plant parts, including roots, and to evaluate the changes in these pools over the growing season as an estimate of diterpenoid alkaloid kinetics in tall larkspur. Twenty entire plants were harvested at each phenological stage: beginning of growth in the spring, early flower, early pod, late pod, and senescence. The plants were separated into their respective parts, freeze-dried, extracted, and analyzed for toxic and total alkaloid concentration, and alkaloid pools were calculated. Concentration of toxic and total alkaloids in leaves and stems declined as the plants matured, while concentration in flowers and pods increased ($P < 0.004$). Concentration of alkaloids in the root declined in the early growth, then increased at the end of the season ($P = 0.002$). Alkaloid pools in the root decreased during early growth, with a corresponding increase of pools in foliar parts. In the late flower and pod stage, alkaloid pools in the leaves and stems declined rapidly, while the pool in the crown and roots tended to increase.

Key Words—Tall larkspur, *Delphinium barbeyi*, poisonous plant, diterpenoid alkaloids, methyllycaconitine, alkaloid pools.

INTRODUCTION

Tall larkspur (*Delphinium barbeyi*) is an important poisonous plant on mountain rangelands, causing acute poisoning and rapid death in cattle. There are many diterpenoid alkaloids in larkspur species, but those that contain the *N*-(methylsuccinimido)-anthranilic ester group (MSAL) are most toxic (Manners

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et al., 1995). Concentration of total alkaloids in tall larkspur, as well as the toxic MSAL alkaloids, decline as the plant matures (Williams and Cronin, 1966; Pfister et al., 1994; Ralphs et al., 1997), rendering the plant relatively nontoxic toward the end of the growing season.

Ralphs et al. (2000) proposed the hypothesis that larkspur alkaloids are synthesized in early growth. As the plant continues to grow, the alkaloids are diluted by the increasing biomass, thus accounting for the linear decrease in toxic and total alkaloid concentrations over the growing season. In contrast, alkaloid pools (the total quantity of alkaloids in the stalks) increase in the first 3–4 weeks of growth, level out from weeks 4 to 6 during the flower stage, and then decline precipitously from late flower through the pod stage when the plant has reached its maximum growth. The objective of this study was to measure the concentration and pools of toxic and total alkaloids in larkspur plant parts, including roots, and evaluate the changes in these pools over the growing season as an estimate of diterpenoid alkaloid kinetics in tall larkspur.

METHODS AND MATERIALS

Tall larkspur plants were harvested from the mountains of central Utah 46 km west of Ferron at an elevation of 3150 m. The site was in the subalpine zone and the vegetation consisted of scattered subalpine fir stands interspersed in the tall forb plant community dominated by tall larkspur. Tall larkspur is a large, robust native perennial plant growing up to 1–2 m tall. The plant consists of many stalks (10–150) growing from the root crown each year.

There is a large amount of variation in concentration of toxic and total alkaloids among tall larkspur plants in a population. Manners and Pfister (1996) suggested 20 tall larkspur plants must be sampled to obtain an accuracy of 10% of the mean at 95% probability. The variability in MSAL alkaloids in tall larkspur plants on our site would require 12–34 plants to be sampled to estimate the population at that level of accuracy and precision (Ralphs et al., 2002). Therefore, we selected 20 plants to dig up at each of the following growth stages: new growth, early flower, early pod, late pod, and senescence. The root mass consists of a caudex of intertwining and connecting tap roots that descend about 25 cm, and then give way to fibrous adventitious roots. The soil was shaken off, and the roots were later washed. Stalks were cut from the root crown at the soil level. Flowering racemes (flower and pod) were removed from the entire plant and placed in a plastic bag. The large biomass of the foliage was unwieldy, and therefore it was subsampled. The wet weight of all the stalks on a plant was recorded, and then three representative stalks were selected and the leaves separated from the stems. Both fresh and dry weights of the leaves and stems were measured and then backcalculated to estimate the dry weight of all the stalks from the entire plant.

The parts (root, stem, leaf, flower, pod) were placed in separate plastic bags and frozen.

At the end of the growing season in late September following senescence, 20 larger plants were dug and the roots harvested. The new growth buds that form next years stalks were cut out from the remainder of the root material to determine if the alkaloid concentration was higher in this new meristematic tissue than the surrounding root tissue. The new buds were white, 1–2 cm in length, and there were 10–40 on each plant. Larger plants were selected to increase the amount of bud tissue for analysis.

Parts from each plant were placed in separate plastic bags, frozen in the field, and freeze-dried. The plants were weighed to obtain dry weight, and then ground through a cyclone grinder to pulverize the material. Alkaloids were extracted and concentrations of toxic and total alkaloids were measured by Fourier-transform infrared spectroscopy (FTIR) (Gardner et al., 1997).

The quantity of toxic and total alkaloid pools in each part was calculated by multiplying the alkaloid concentration by the dry weight of that part. Alkaloid pools are used here to express the amount of alkaloids in a plant part, which better describes the dynamics than does alkaloid concentration.

The concentration and pools of MSAL alkaloids were compared in a repeated-measures mixed model analysis of variance, comparing plant parts and dates of collection using the compound structure as a covariate. There was a significant part-by-date interaction; therefore, the model was reduced, and alkaloids in each part were compared over time. Orthogonal contrasts were used to describe the response of each part over time.

RESULTS

Alkaloid Concentration. Toxic MSAL alkaloid concentrations differed among all the larkspur plant parts ($P < 0.001$). MSAL concentration was highest in the early leaves at the beginning of the study and declined linearly over the season ($P < 0.001$) (Figure 1). Concentrations of MSAL alkaloids in stems were similar to the leaves and declined linearly ($P < 0.001$). The floral parts increased slightly at the end of the season ($P = 0.004$). Concentrations of MSAL alkaloids in the roots declined during the flower stage and then increased toward the end of the season in a quadratic manner ($P = 0.002$).

Toxic alkaloid concentration in the new growth root buds at the end of the season was lower than in the remainder of the root tissue (5.7 ± 0.33 vs. 14.9 ± 1.3 mg/g, $P < 0.001$). This was contrary to plant defense theory, which states that new meristematic tissue should be higher in defense compounds (McKey, 1974). However, as these new shoots begin rapid aerial growth in the spring, they have high concentrations of toxic and total alkaloids (Figure 1).

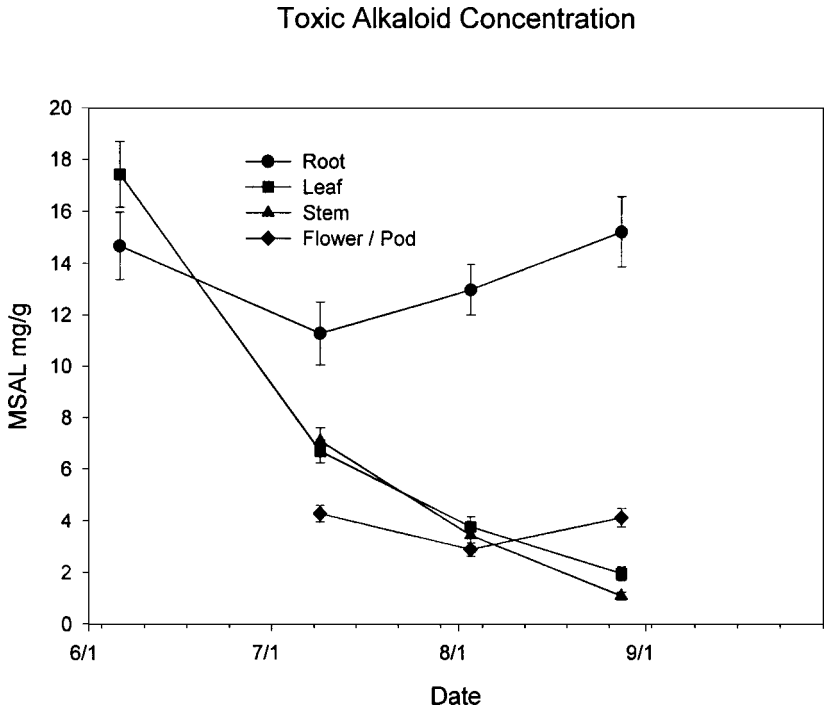

FIG. 1. Toxic alkaloid concentration in larkspur plant parts. Bars are standard error of the mean.

Alkaloid Pools. MSAL alkaloid pools in roots were greater than in the leaf and stem ($P < 0.001$; Figure 2). Although the concentration of toxic alkaloids in the roots differed over the season ($P = 0.002$), the alkaloid pools in roots were not significantly different ($P = 0.37$), due to the large variation in root size (see wide standard error bars of roots in Figure 2). However, the direction of change indicates that alkaloid pools declined in the flower stage, and then increased in the pod stage (Figure 2).

The change in the toxic alkaloid pool in leaves and stems was opposite to that of the roots. There was a significant difference ($P < 0.001$) over the season, and the concave quadratic response ($P < 0.001$) indicates the pool in leaves and stems increased from the vegetative to the flower stage, and then declined during the pod stage (Figure 2). The small pools in the floral parts (due to the relatively small biomass) increased from the flower to the pods ($P = 0.002$).

The quantitative change in MSAL alkaloid pools in larkspur plant parts (Table 1) shows that the relative increase in larkspur leaves and stems ($+273$ mg) is greater than the decline in roots (-172 mg) during the vegetative stage of growth.

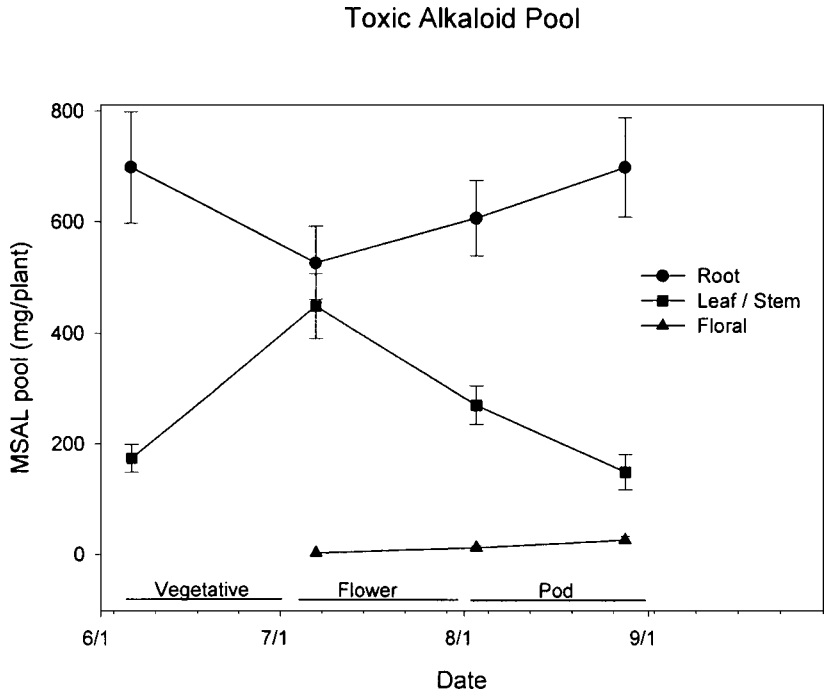

FIG. 2. Toxic alkaloid pools in larkspur plant parts. Bars are standard error of the mean.

The difference in magnitude may be due to continued alkaloid synthesis in roots in early growth (Ralphs et al., 2000). The loss of alkaloids from the leaf/stem (−292 mg) during the flower and pod stages was greater than the gain in the roots (+173 mg) at the end of the season. This suggests there may be some catabolism of the alkaloids. The seed pool is relatively small (26 mg), and would not account for much loss of alkaloid as the seed is shed. It is unlikely that toxic alkaloids are

TABLE 1. CHANGE IN MSAL ALKALOID POOLS IN LARKSPUR PLANT PARTS OVER THE GROWING SEASON

		Root		Leaf/stem		Flower/pod	
Date	Stage	Pool	Change	Pool	Change	Pool	Change
				mg			
6/6	Veg	698	−172	174	+273		
7/9	Flower	526	+81	447	−177	2	+8
8/3	Pod	607	+92	270	−121	12	+14
8/29	Seed ripe	699		149		26	

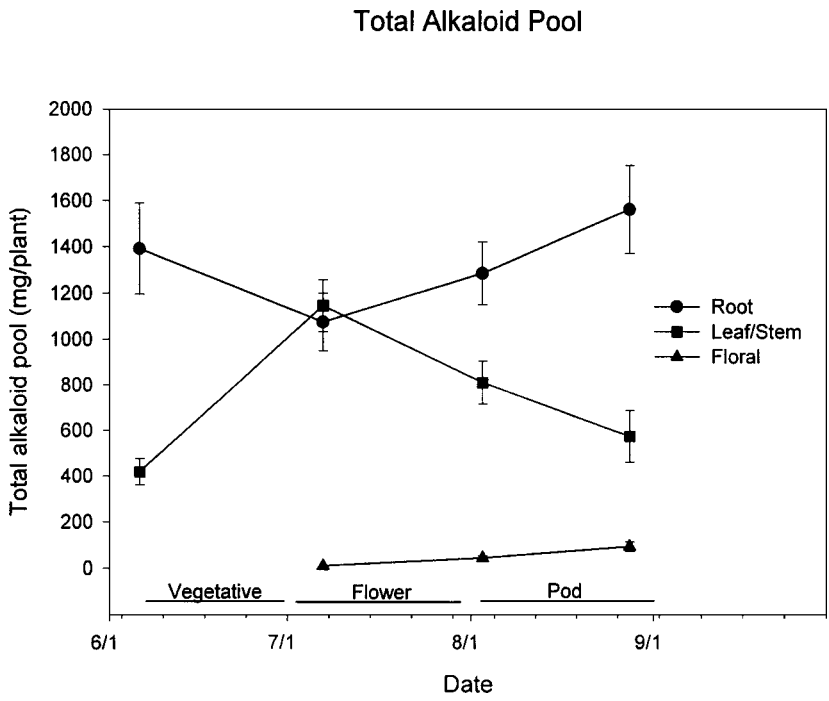

FIG. 3. Total alkaloid pools in larkspur plant parts. Bars are standard error of the mean.

metabolized to other alkaloids since the total alkaloid pools responded in a similar fashion (Figure 3).

DISCUSSION

Alkaloids are classified according to their biosynthetic origin. Diterpenoid alkaloids are characterized by the structural fragment derived from the C_{20} terpenoid. Norditerpenoid alkaloids are partially degraded structures. Biosynthetic pathways for norditerpenoid alkaloids have been postulated; however, experimental evidence is lacking (Joshi and Pelletier, 1999). It is assumed that norditerpenoid larkspur alkaloids are derived from the mevalonate pathway of isoprenoid synthesis (Cordell, 1981). However, another pathway for terpenoid synthesis has recently been proposed, starting from pyruvate and glyceraldehyde-3-phosphate (Lichtenthaler et al., 1997).

The biosynthesis of monoterpenes in peppermint (*Mentha piperita*) has been described by Gershenzon (1994) and presents a model similar to what we have observed in larkspur. Monoterpenes were produced only during the first 2 weeks

of growth when enzymes for synthesis were present in the plant. The amount of monoterpenes remained constant for the remainder of the growth stage, but 50–75% of the monoterpenes were catabolized after the plants began to flower (Croteau, 1988). The principal monoterpene, menthone, was reduced to equal amounts of menthol and neomenthol. Neomenthol was transported via its glycoside to the rhizome where it was sequentially hydrolyzed and oxidized to reform menthone (Croteau et al., 1984).

Hartman et al. (1989) reported that pyrrolizidine alkaloids (PA) in *Senecio vulgaris* were synthesized in roots, and then translocated up to the shoot through the phloem and stored mostly in the inflorescence. The alkaloids were synthesized and translocated as the N-oxide, which is a polar, salt-like compound that is soluble in water and suited for transport. However, almost 70% of the total amount of alkaloid was lost during the period of flowering to senescence, and most of that remaining in the plant was in the seed (Hartman and Zimmer, 1986).

The kinetics of alkaloid pools in tall larkspur in our study is similar to that of monoterpenes in peppermint and PA in Senecio. Benn and May (1964) suggested that biosynthesis of diterpenoid alkaloids occurs in larkspur roots. Strzelecka (1968) also reported that methyllycaconitine (MLA), the major MSAL alkaloid, was synthesized in root cultures of *D. elatum*. Environmental stresses, such as light, temperature, and photosynthesis inhibition, did not affect toxic or total alkaloid pools in tall larkspur (Ralphs et al., 1998). Since these major factors that control plant metabolism did not affect alkaloid pools, we speculate that alkaloids are not synthesized in the leaves. The decline of alkaloid pools in tall larkspur roots and the corresponding increase in the foliar parts suggests the alkaloids are being translocated upward in the early growth.

The decline in tall larkspur alkaloid pools in the above ground parts following flowering is consistent with other species, such as peppermint and *Senecio*. Kreps (1969) reported that concentration of total alkaloids in the root crown of duncecap larkspur increased slightly in late summer when the buds on the crown began to enlarge. Strzelecka (1967) reported that MLA accumulated in roots and rhizomes of *D. elatum* during the flower stage and as new buds form on rhizomes. However, it is not known if the alkaloids are translocated back to the root, or if they are catabolized in the shoots, and synthesis in the root increases at this time. It is peculiar that the concentration of alkaloids in the root buds in our study were substantially lower than in the surrounding root tissue in the fall.

In summary, alkaloid pools in tall larkspur declined in roots during early growth, while pools in foliar parts increased. We speculate that the alkaloids are synthesized in the roots and are translocated upward to the leaves and stems. During the late flower and pod stage, pools in the leaves and stems declined rapidly, while pools in roots increased. However, we do not know if the alkaloids are translocated back to the roots, or if they are catabolized in the shoots and synthesis in roots began anew. A relatively small amount of alkaloid is lost in the seeds.

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